

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule encoding an isopentenyl diphosphate pathway enzyme, selected from the group consisting of:
 - 5 (a) an isolated nucleic acid molecule encoding the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13;
 - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS at 65 °C, and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
 - 10 (c) an isolated nucleic acid molecule that is completely complementary to (a) or (b).
2. The isolated nucleic acid molecule of Claim 1 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.
- 15 3. A polypeptide encoded by the isolated nucleic acid molecule of Claim 1.
4. The polypeptide of Claim 3 selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13.
- 20 5. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding an acetyl-coA acetyltransferase enzyme that has at least 65% identity over length of 411 amino acids based on the CLUSTALW method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:8 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
- 25 6. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding an HMG-coA synthase enzyme that has at least 82% identity over length of 464 amino acids based on the CLUSTALW method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:9 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
- 30 7. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a mevalonate kinase enzyme that has at least 68% identity over length of 386 amino acids based on the CLUSTALW method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:11 or a second nucleotide sequence comprising the

complement of the first nucleotide sequence.

8. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding an phosphomevalonate kinase enzyme that has at least 73% identity over length of 503 amino acids based on the

5 CLUSTALW method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:12 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

9. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a mevalonate diphosphate decarboxylase enzyme 10 that has at least 77% identity over length of 415 amino acids based on the CLUSTALW method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:13 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

10. A chimeric gene comprising the isolated nucleic acid molecule 15 of any one of Claims 1 or 5-9 operably linked to suitable regulatory sequences.

11. A transformed host cell comprising the chimeric gene of Claim 10.

12. The transformed host cell of Claim 11 wherein the host cell is 20 selected from the group consisting of bacteria, yeast, filamentous fungi, algae and green plants.

13. The transformed host cell of Claim 12 wherein the host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Salmonella*, *Bacillus*, 25 *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Flavobacterium*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Escherichia*, *Erwinia*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, 30 *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium* and *Klebsiella*.

14. The transformed host cell of Claim 12 wherein the host cell is selected from the group consisting of rubber tree (*Hevea brasiliensis*), 35 tobacco (*Nicotiana* spp.), tomato (*Lycopersicon* spp.), potato (*Solanum* spp.), hemp (*Cannabis* spp.), sunflower (*Helianthus* spp.), sorghum (*Sorghum vulgare*), wheat (*Triticum* spp.), maize (*Zea mays*), rice (*Oryza sativa*), rye (*Secale cereale*), oats (*Avena* spp.), barley (*Hordeum vulgare*), rapeseed (*Brassica* spp.), broad bean (*Vicia faba*), french bean

(*Phaseolus vulgaris*), other bean species (*Vigna* spp.), lentil (*Lens culinaris*), soybean (*Glycine max*), arabidopsis (*Arabidopsis thaliana*), guayule (*Parthenium argentatum*), cotton (*Gossypium hirsutum*), petunia (*Petunia hybrida*), flax (*Linum usitatissimum*) and carrot (*Daucus carota sativa*).

5 15. A method of obtaining a nucleic acid molecule encoding an isopentenyl diphosphate pathway enzyme comprising:

- (a) probing a genomic library with the nucleic acid molecule of any one of Claims 1 or 5-9;
- 10 (b) identifying a DNA clone that hybridizes with the nucleic acid molecule of any one of Claims 1 or 5-9; and
- (c) sequencing the genomic fragment that comprises the clone identified in step (b),

wherein the sequenced genomic fragment encodes an isopentenyl diphosphate pathway enzyme.

15 16. A method of obtaining a nucleic acid molecule encoding an isopentenyl diphosphate pathway enzyme comprising:

- (a) synthesizing at least one oligonucleotide primer corresponding to a portion of the sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6; and
- 20 (b) amplifying an insert present in a cloning vector using the oligonucleotide primer of step (a);

wherein the amplified insert encodes a portion of an amino acid sequence encoding an isopentenyl diphosphate pathway enzyme.

25 17. The product of the method of Claims 15 or 16.

18. A method for the production of a compound in the isopentenyl diphosphate pathway comprising: contacting a transformed host cell under suitable growth conditions with an effective amount of a carbon substrate whereby a compound in the isopentenyl diphosphate pathway is produced, said transformed host cell comprising a set of nucleic acid molecules encoding SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6 under the control of suitable regulatory sequences.

30 35 19. A method according to Claim 18 wherein the transformed host is selected from the group consisting of bacteria, yeast, filamentous fungi, algae and green plants.

20. A method according to Claim 19 wherein the transformed host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Flavobacterium*,
5 *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Escherichia*, *Erwinia*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium* and
10 *Klebsiella*.

21. A method according to Claim 19 wherein the transformed host cell is selected from the group consisting of rubber tree (*Hevea brasiliensis*), tobacco (*Nicotiana* spp.), tomato (*Lycopersicon* spp.), potato (*Solanum* spp.), hemp (*Cannabis* spp.), sunflower (*Helianthus* spp.),
15 sorghum (*Sorghum vulgare*), wheat (*Triticum* spp.), maize (*Zea mays*), rice (*Oryza sativa*), rye (*Secale cereale*), oats (*Avena* spp.), barley (*Hordeum vulgare*), rapeseed (*Brassica* spp.), broad bean (*Vicia faba*), french bean (*Phaseolus vulgaris*), other bean species (*Vigna* spp.), lentil (*Lens culinaris*), soybean (*Glycine max*), arabiopsis (*Arabidopsis thaliana*), guayule (*Parthenium argentatum*), cotton (*Gossypium hirsutum*), petunia (*Petunia hybrida*), flax (*Linum usitatissimum*) and carrot (*Daucus carota sativa*).

22. A method of regulating isopentenyl diphosphate biosynthesis in an organism comprising, over-expressing at least one isopentenyl diphosphate gene selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6 in an organism such that isopentenyl diphosphate biosynthesis is altered in the organism.
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23. A method according to Claim 22 wherein said isopentenyl diphosphate pathway gene is over-expressed on a multicopy plasmid.
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24. A method according to Claim 22 wherein said isopentenyl diphosphate pathway gene is operably linked to an inducible or regulated promoter.

25. A method according to Claim 22 wherein said isopentenyl diphosphate pathway gene is expressed in antisense orientation.
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26. A method according to Claim 22 wherein said isopentenyl diphosphate pathway gene is disrupted by insertion of foreign DNA into the coding region.

27. A mutated gene encoding an isopentenyl diphosphate pathway enzyme having an altered biological activity produced by a method comprising the steps of:

(i) digesting a mixture of nucleotide sequences with 5 restriction endonucleases wherein said mixture comprises:

- a) a native isopentenyl diphosphate gene;
- b) a first population of nucleotide fragments which will hybridize to said native isopentenyl diphosphate gene;
- c) a second population of nucleotide fragments which will not hybridize to said native isopentenyl diphosphate gene;

10 wherein a mixture of restriction fragments are produced:

- (ii) denaturing said mixture of restriction fragments;
- (iii) incubating the denatured said mixture of restriction fragments of step (ii) with a polymerase;
- (iv) repeating steps (ii) and (iii) wherein a mutated isopentenyl diphosphate gene is produced encoding a protein having an altered biological activity.

28. An isolated nucleic acid molecule encoding an HMG-coA 20 reductase enzyme having nucleotide sequence as set forth in SEQ ID NO:3.